AN ISOLATED PEPTIDE OR POLYPEPTIDE OF THE EXTRACELLULAR PORTION OF THE HUMAN INTERFERON RECEPTOR (IFN-R)

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN: alpha (α), beta (β) and gama. IFNα and IFNβ together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33: 251, 1986).

The human IFNα locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, αII or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFNα genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78: 2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFNβ) is encoded by a single gene which has approximately 50% homology with the IFNα genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

BRIEF DESCRIPTION OF THE DRAWINGS

-- Figure 1: binding of ^{125}I-labelled monoclonal antibodies 34F10 and 64G12 to:

-- A: Daudi cells

-- B: Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold excess of cold antibodies and subtracted from total counts.

-- Figures 2A and 2B: nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in Figures 2A and 2B.

-- Figures 3A and 3B: nucleotide and corresponding amino-acid sequence of the human IFN-R.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of cos cells

| 18 h

serum free medium

|

supernatants taken after 48h, 72h, 96h

F3

|

concentration

|

NTA column

|

| Wash PBS

elution 0.1 M NaOAc pH 5.5

|

| neutralization

concentration, 30 000 cut off

|

|

MONO Q (0-0.5 M Na Cl)

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35: 1-32, 1980). Briefly, $5x10^7$ spleen cells were fused to $3x10^7$ myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as $20x10^7$ spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method:

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.5% TWEEN 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% TWEEN 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.